Denitrification in created riverine wetlands: Influence of hydrology and season

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Abstract

Seasonal denitrification rates in two created riparian marshes were investigated under pulsing and steady-water flow conditions. Denitrification was measured using the in situ acetylene block technique. Measurements were performed in a transverse gradient with different hydrologic conditions: low marsh and open water zones which were permanently flooded, high marsh zones which had permanently saturated soils but standing water during pulses, and edge zones which were normally dry with standing water during flood pulses. Denitrification in all plots was significantly correlated with soil temperature and was significantly correlated with the nitrate concentration in the inflow surface water in the growing season. Late spring denitrification rates in the high marsh zone were significantly higher under flood pulsing (778 ± 92 mg N m^{-2} h^{-1}) than under steady flow (328 ± 63 mg N m^{-2} h^{-1}). In the low marsh and edge zones, flood pulses did not affect denitrification. N_2O/N_2 ratios were higher in intermittently flooded (high marsh and edge) zones than in permanently flooded (low marsh) zones and highest in the cold seasons. Highest mean denitrification rates were observed in the low marsh zone (800 ± 102 mg N m^{-2} h^{-1}) and they were significantly higher ($P < 0.05$) than in the high marsh (458 ± 87 mg N m^{-2} h^{-1}) and edge (315 ± 40 mg N m^{-2} h^{-1}) zones but not significantly different from the open water zone (584 ± 101 mg N m^{-2} h^{-1}). Denitrification in high marsh zones was not significantly different than in the open water and edge zones. In permanently flooded areas, denitrification rates were significantly higher near the wetland inflow than near the outflow. Overall, denitrification in the experimental wetlands was 147 ± 54 kg N yr^{-1} during pulsing year and 112 ± 41 kg N yr^{-1} during steady-flow. Denitrification appeared to be nitrogen limited in low marsh, high marsh and edge plots, but both carbon and nitrogen limited in open water.

Introduction

Agricultural runoff is a main source of nitrogen loading in the Mississippi River and increases of this nitrate loading is cited as the major cause of the extensive hypoxia in the Gulf of Mexico (Goolsby and Battaglin, 2001; Dagg and Breed, 2003). To mitigate this problem, the creation and restoration of wetlands has been recommended in the Mississippi–Ohio–Missouri (MOM) river basin (Mitsch et al., 2001, Mitsch et al, 2005a, Mitsch and Day 2006). Nitrogen in wetlands is removed from the water by biological transformations. Plant uptake and microbes temporary immobilize nitrogen, whereas permanent nitrogen removal occurs via denitrification (Poe, et al, 2003; Clement et al, 2002). Denitrification is the reduction of NO_3- to nitrogen gaseous forms such as N_2O and N_2; this process is carried out by anaerobic facultative bacteria in anoxic conditions. Denitrification is controlled by oxygen availability, temperature, nitrogen and organic carbon supply (Beauchamp, et al., 1989). While several studies have investigated how these controlling factors affect denitrification rates in riparian buffer zones (Ellman et al, 2004, Ambus and Lowrance, 1991; Clement et al., 2002, Willems, et al, 1997; Matheson, et al, 2003, Martin, et al, 1999), few studies have investigated denitrification in created wetlands receiving non point source pollution or river flood water (Poe, et al, 1993, Srivedhin and Gray, 2006).

Created or restored riverine wetlands are expected to experience flood pulsing. Flooding facilitates the exchange of material between rivers and their floodplains (Junk et al., 1989). The reestablishment of flood pulsing in riverine and tidal systems is being recognized as an essential step in the restoration of wetlands (Middleton, 2002). Flood pulses are also nutrient pulses and they often make the wetland area larger, changing the oxygen availability of soils and the potential area for denitrification to occur. The effect of flood pulses on nitrogen cycling in created riverine wetlands is not completely understood. In a longitudinal gradient, i.e., along the water flow, a decrease in nitrate concentrations is expected. On the other hand, in a transverse gradient, soils have different flooding frequencies and therefore oxidative–reductive conditions. Since nitrate and oxygen availability are key factors controlling denitrification (Ellman et al, 2004, Beauchamp, et al., 1989) it is expected that denitrification rates vary along these gradients.

High nitrogen removal in riverine wetlands created or restored for controlling agricultural nitrate loads to rivers is desirable and denitrification is a desirable mechanism for nitrogen removal because the bacterial conversion to gaseous forms permanently removes nitrogen from the watershed. Thus, quantifying and understanding this process in created wetlands is important for scientists and managers seeking to create long-term improvement of water quality.

The objectives of this study were to investigate seasonal denitrification rates in zones in longitudinal and transverse gradients in two similar 1-ha created wetlands in Midwestern

USA under both pulsing and steady-flow conditions, and to assess the controlling factors of denitrification in these zones. Hydrology in these wetlands was completely controlled, giving the opportunity to design experiments with different hydrologic regimes.

Material and Methods

Site description and hydrologic experiment

This study was conducted at the Schiermeier Olentangy River Wetland Research Park (ORWRP) in Columbus, Ohio, USA (latitude N 40.021°, longitude E 83.017°). The ORWRP includes several wetlands that are flooded with different waters and at different frequencies. Our study was carried out in a pair of 1-ha experimental river-diversion wetlands created on alluvial old-field soils adjacent to the third-order Olentangy River in 1993–94. Both wetlands have three deepwater (>50 cm depth) sections located in the inflow, middle and outflow positions of the basins, surrounded by much shallower sections (20–30 cm deep) dominated by emergent plants. The hydrology in these wetlands is mostly controlled by river water pumped from the Olentangy River. Water enters to these wetlands at their north side, flows southwards through the wetland, and finally returns to Olentangy River through an outflow swale (Figure 1). The primary original soil type at the experimental wetlands is a Ross (Rs) series soil, which is a floodplain alluvial soil that ranges from silt loam to silty clay loam to loam (Mitsch and Wu, 1993). These wetland basins were artificially flooded for 10 years prior to the start of this study and had developed hydric soils over that time (Mitsch et al., 2005c; Anderson et al., 2005). The biogeochemistry and ecology of these wetlands has been described in several other publications (Mitsch et al., 1998, 2005a,b,c; Nairn and Mitsch, 2000; Spieles and Mitsch, 2000; Harter and Mitsch, 2003; Anderson et al., 2005; Anderson and Mitsch, 2006; Hernandez and Mitsch, in press; Altor and Mitsch, in final review).

The study period was from May 2004 to December 2005. The wetlands were treated as replicates, receiving the same amount of water under two different hydrologic conditions (pulsing and steady flow). In spring 2004, the wetlands received controlled seasonal hydrologic pulses, and during 2005 they received a steady rate of water inflow. Seasonal hydrologic pulses were simulated by pumping river water at high rates (27–54 cm d^{-1}) during the first week of each month; during the remaining three weeks of the month the wetlands received a low flow rate (11 cm d^{-1}). The pulse flow schedule operated from January through June to simulate winter/spring flooding. From July to December the wetlands received a steady non-pulsing flow. There were also two natural flooding events of these floodplain wetlands by the Olentangy River: on June 14, 2004, and January 4, 2005. An estimated equal amount of flooding occurred in each wetland during these events.

Gas sampling protocol

To evaluate the effect of hydrological pulses on denitrification, measurements were taken in zones at different elevations above mean water level (221.10 m AMSL) where the flood frequency would be affected by flood pulses. The edge zone was at +0.18 m, the high marsh zone at 0.03 m, the low marsh zone at -0.09 m, and the open water zone at -0.38 m (Figure 1). The edge zone was usually dry with standing water during flood pulses, the high marsh zone was saturated with alternate standing water and air exposed conditions, and the low marsh and open water zones were permanently flooded. During the pulse year (2004), denitrification was measured from May to December; the frequency of measurements was two times in May, three times in June and once per month for the rest of the year. During the steady flow conditions (2005), denitrification was measured once per month from January to April, and in the following months, measurements were made at the same frequency as in 2004. Denitrification in open water zones was measured from August 2004 to November 2005, with the same frequency described above, but due to a thick layer of ice, sampling in these plots was not possible in December 2004 or January, February, and December 2005. Because the open water zone had hydrologic conditions similar to the low marsh (permanently flooded), denitrification rates in this zone were not investigated during flood pulsing. Thus, due to the fact that we had fewer measurements in this area, denitrification rates in the open water zone were only included in the longitudinal spatial analysis. For uniformity, all samples were taken between 11:00 am and 3:00 pm.

Measurement of denitrification in situ

The acetylene inhibition technique was utilized to measure denitrification. Acetylene inhibits the reduction of N_2O to N_2, during denitrification. Production of N_2O in the presence of acetylene is equivalent to production of N_2O plus N_2 in the absence of acetylene. Variations of this technique include either 1) in situ treatment of soil with acetylene, followed by determination of N_2O emissions, or 2) incubations of soil cores with acetylene followed by N_2O analysis (Knowles, 1990). We evaluated the advantages and disadvantages of using the two approaches of the acetylene technique. Because we were interested in the effects of hydrologic dynamics on denitrification, the incubation of soil cores had constraints. For example, taking cores frequently would cause high disturbances in our plots, and when the plots were inundated, getting an intact soil core sample without losing its water content would be difficult. To minimize acetylene resistance or nitrification inhibition from repeated acetylene exposure (Mosier et al., 1986), measurements were randomly taken within a 0.50 m^2 area to avoid repeated acetylene application in the same sampling plot.

We measured total denitrification ($N_2 + N_2O$ production) adapting the acetylene inhibition technique in the field described by Ryden and Dawson (1982). We used PVC

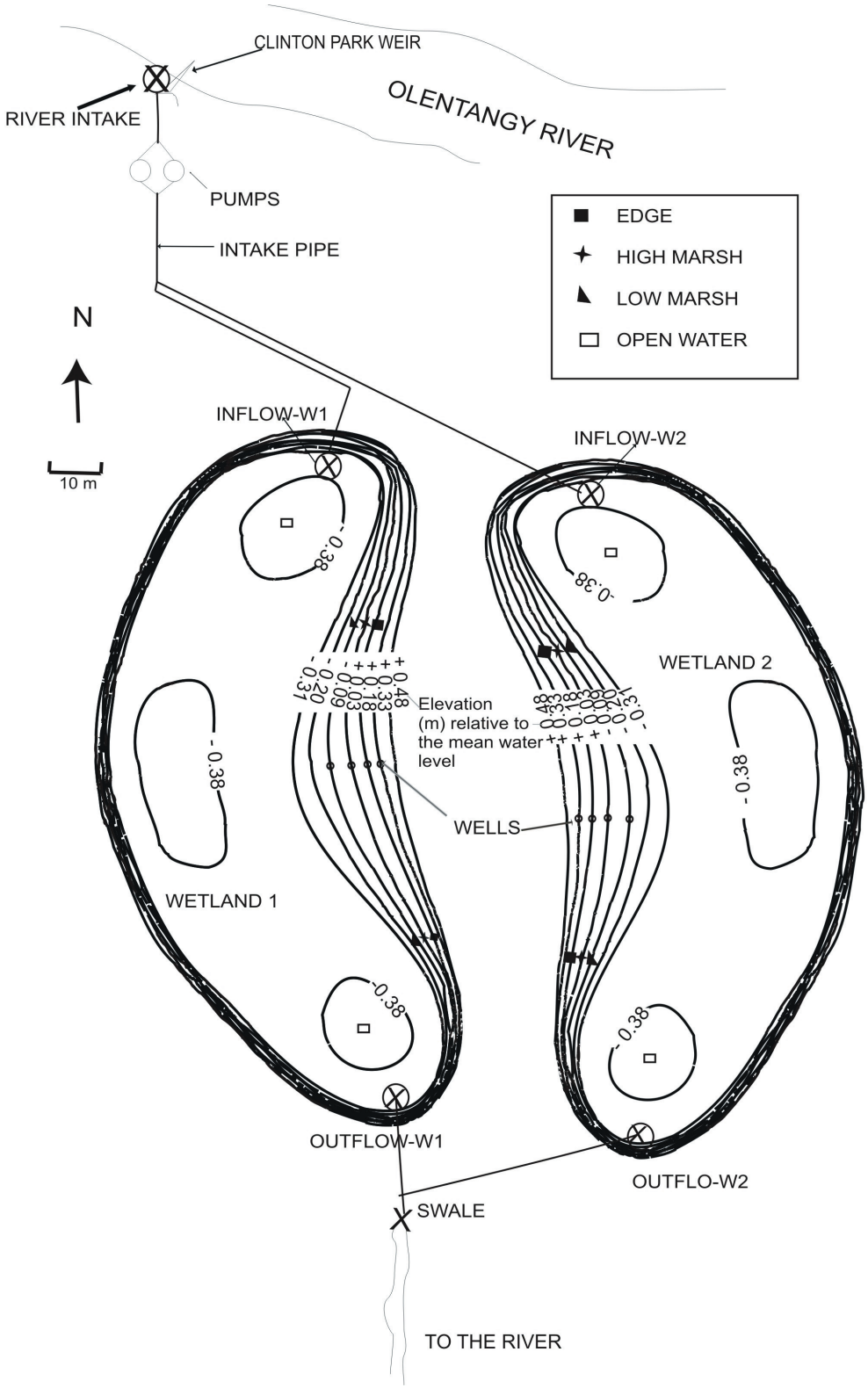

Figure 1. Two 1-ha experimental wetlands at Olentangy River Wetlands Research Park (ORWRP), The Ohio State University, Columbus, OH, USA, used in this study. Sample locations on gradients on inside of kidney-shaped wetlands are indicated. Circular and oval areas in each wetland are deepwater basins. Contours are shown in meters above mean water level.

chambers (4-cm diameter x 75-cm high), with a collar in the upper part to hold water for sealing purposes. They were placed 10 cm into the soil 24 h before gas measurements. Acetylene was injected 10 cm into the soil using a perforated PVC pipe (4 mm i.d.) to obtain a final concentration of 10% v/v in the headspace. Thirty minutes after acetylene injection, the chambers were closed with a cap (4-cm tall) with a thermometer, pressure vent, and gray butyl rubber sampling port, and were sealed using water. Gas samples were taken every 10 minutes during a 30 minute period, were transferred to an evacuated 20 ml Wheaton bottle, capped with a rubber stopper and an aluminum seal, stored in a refrigerator at 4°C, and analyzed within four days. The period before gas sampling was established in an experiment at the beginning of our study. The experiment consisted of measurements of N_2O fluxes in all plots immediately after acetylene application and 15, 30, 45 and, 60 minutes after application. N_2O fluxes immediately after acetylene application were not linear in any of the plots; after 15 minutes fluxes in the edge zone were linear and after 30 minutes, linear fluxes were observed in all plots. Therefore, 30 minutes was established as the period for acetylene diffusion.

Total denitrification was measured after quantification of N_2O fluxes without acetylene. Details of methodology for measuring N_2O fluxes without acetylene are described in Hernandez and Mitsch (in press).

Water level, soil and water temperature were measured in the plots each time that denitrification was measured. When surface water was present in the plots, water level was measured using a meter stick. When no surface water was present, water level was recorded in shallow PVC wells using a Solinst measuring tape. There was one well for each elevation (high marsh and edge zone) in the middle of each wetland (Figure 1). Soil temperature was measured at 5 and 10 cm from the surface during each sampling event using a soil thermometer probe meter (Fluke 51 II). Water temperature was measured 3 cm below the surface with an alcohol-type thermometer.

Role of carbon and nitrogen as limiting factors

To determine if carbon or nitrogen were the factors controlling denitrification, soil cores (4 cm diameter x 9 cm depth) were collected in June 2005 in the same plots where in situ denitrification was measured. Denitrification potential was measured in the soil slurries using the acetylene block technique described by Tiedje (1982). Two cores were taken in each plot and a portion of one core was used for bulk density analysis; the remainder of that core and the second core were homogenized by hand, roots and twigs were removed, and in this homogenized soil, physicochemical analysis and incubations were performed. For details on methodology and results of psychochemical analysis see Hernandez and Mitsch (in press). Samples of homogenized fresh soil (approximately 15 g dry weight) were placed in 1000 ml Manson jars; each jar had a gray butyl septum for gas sampling and a 15 cm sealable vent tube (tygon 2 mm i.d.) attached to the lid. Each soil sample had four treatments: 1) 50 ml of distilled water, 2) 50 ml of 200 mg L^{-1} of N as KNO_3 solution, 3) 2 g L^{-1} of glucose-C solution, and 4) 50 ml of 200 mg L^{-1} of N and 2 g L^{-1} of glucose-C solution. Each treatment was carried out by triplicate. Jars were closed and flushed with oxygen-free N_2 for two minutes at a flow rate of 8 L min^{-1}; this was done to provide anoxic conditions. While the jars were flushed with N_2, the tygon tube was open and submerged in water; when flushing was finished, it was closed with a small clamp and 10% of the volume was replaced by acetone free acetylene. The slurries were incubated at 20 ± 3°C, and headspace gas was sampled at 0, 6, 12, 24 and 30 hours. The jars were shaken by hand approximately every 3 hours, and before the gas sampling.

Analytical methods

Gas analysis

Nitrous oxide was analyzed using a gas chromatograph (Shimadzu GC-14-A) fitted with a 2 ml sampling loop, two Porapak-Q 1.8 m columns and an electron capture Ni-63 detector. For field data, total denitrification rates were calculated from linear nitrous oxide production in acetylene presence using the closed chamber flux equation (Holland et al., 1999). For incubations, denitrification potential was calculated from the linear portion of a graph of N_2O produced vs. sampling time. Gas concentrations measured in the headspace were adjusted for the gas in solution using the Bunsen solubility coefficient (Tiedje, 1982).

Water analysis

Weekly surface water samples were taken at inflow, middle, and outflow locations in the wetlands for nutrient analysis. Samples were acidified and kept at 4°C until they were analyzed. Nitrate + nitrite was analyzed by the sulfanilamide method after reduction in a cadmium column and ammonia was analyzed by the phenolate method. Both methods were adapted for use in a Flow Injection Lachat QuikChem IV Autoanalyzer (Lachat, 2000). Ammonia concentrations were below the detection level of 0.01 mg-N L^{-1}.

Statistical analysis

Statistical analyses were performed with SPSS version 11 for Macintosh and version 12 for Windows. Kolmogrov-Smirnov, Lilliefors' and Shapiro-Wilk's tests were used to check normality. In several cases, denitrification rates measured in situ did not follow a normal distribution and they could not be transformed to fit a normal distribution. Therefore, they were analyzed using nonparametric techniques. The Mann-Whitney u-tests were used to check significance of differences among transverse and longitudinal gradients and differences under pulsing versus steady flow conditions. Relationships between denitrification, soil temperature, and water nitrate concentration were examined using the Spearman Rank Order correlation. Results from denitrification potential measured under lab conditions fit normal distribution; therefore one-way analysis of variance

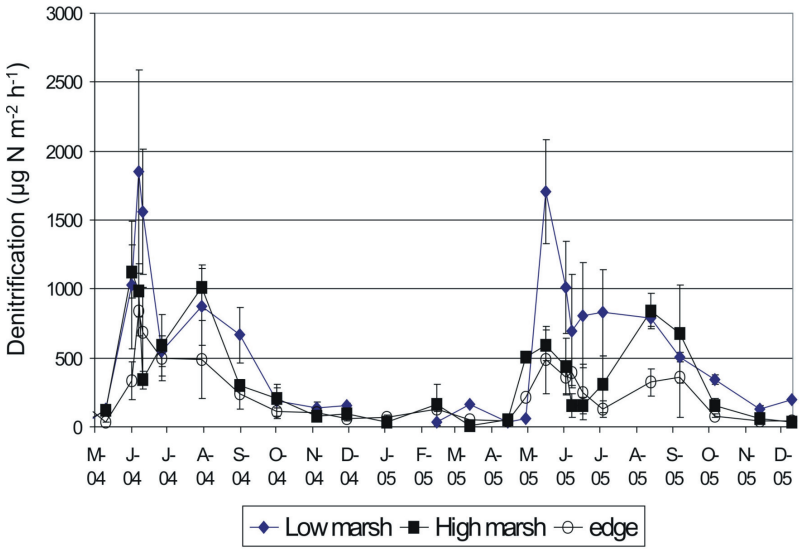

Figure 2. Seasonal denitrification rates in zones with different flood frequency in created riverine wetlands during the study period May 2004–December 2005.

(ANOVA) with Tukey HSD multiple comparison tests was used to detect differences among the treatments. A 5% significance level was used to assess differences among treatments.

Results

Seasonal patterns

Denitrification rates in these created wetlands (Figure 2) were strongly affected by the hydrologic conditions in the plots, soil temperature, and nitrate concentration in surface water (Figures 3a, b and c). During 2004, the low marsh plots, which were permanently inundated, showed the highest mean denitrification rates (1850 ± 735 mg N m^{-2} h^{-1}) in early June when the highest nitrate concentration (4.1 mg L^{-1}) in the inflow surface water was observed and soil temperatures were between 21–23°C. Under steady flow conditions in 2005, these plots showed the highest mean denitrification rate (1707 ± 378 mg N m^{-2} h^{-1}) in late May when the highest mean nitrate concentration in the inflow surface water (4.20 mg L^{-1}) was observed and soil temperatures oscillated between 24–25°C. The high marsh plots, which were flooded during pulses and had saturated soils under steady flow conditions, showed a different pattern; in 2004 (pulsing year) the highest mean denitrification rates were observed during the flood pulses of June (1125 ± 463 mg N m^{-2} h^{-1}) when soil temperatures were 19–21°C. In 2005 (steady-flow year), highest denitrification (841 ± 131 mg N m^{-2} h^{-1}) was in August when soil temperatures were 27–28°C. In 2004, edge plots, which were flooded during pulses and dry under steady flow conditions, showed highest denitrification rates (836 ± 177 mg N m^{-2} h^{-1}) in June when they were inundated and soil temperatures were 19-20°C. In 2005, the highest mean denitrification rates (538 ± 245 mg N m^{-2} h^{-1}) in the edges zone were observed in May when soil temperatures

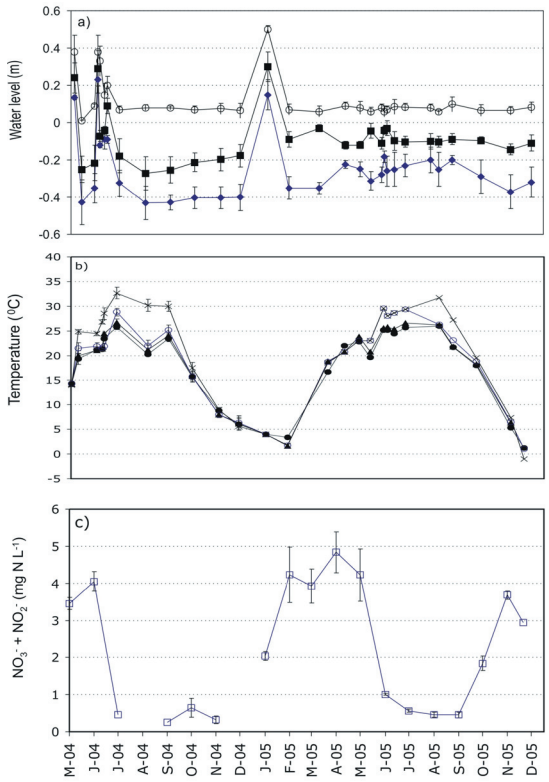

Figure 3. Seasonal dynamics of (a) water level, (b) soil and water temperature, and (c) surface water nitrate concentration in the inflow at different elevations in the experimental wetlands at ORWRP. Values for (a) and (b) are means of two plots in each wetland (n = 4). When no surface water was present, water level results are the means of two measurements in each elevation (n = 2). Values for (c) are mean of weekly samples (n = 4).

Table 1. Spearman's correlation coefficient between denitrification, soil temperature and nitrate concentration in the inflow surface water.

	Denitrification rates		
	Low marsh	High marsh	Edge
Temperature	0.579**	0.608**	0.596**
Nitrate inflow surface water	0.231	0.231	-0.007
Nitrate inflow surface water (May-September)	0.747**	0.511	0.421

**Significant at 0.05 probability level

Table 2. Area of the different hydrologic zones in each kidney-shaped created experimental wetland at the Olentangy River Wetland Research Park.

	Area (m^2)
Open water	4,096*
Low marsh	6,113
High marsh	2,365
Edge	2,709
Area with standing water during flood pulses	15,283
Area with standing water during steady flow conditions	10,170

* Source Mitsch and Zhang (2004)

were 19–21°C. Lowest denitrification rates were observed in fall and winter of both years, for all plots.

A significant relationship between denitrification rates and soil temperature was found in all different hydrologic zones (Table 1). However, no significant relationship between denitrification rates and nitrate concentration in the inflow surface water was found in any of the plots. When denitrification rates obtained during the warm season (May–September) were correlated with the nitrate concentration in surface water, a significant correlation was found only in the low marsh (Spearman's correlation coefficient = 0.747).

Effect of hydrologic pulses on seasonal denitrification rates in the transverse gradient

Denitrification rates in low marsh plots were not affected by flood pulses; these plots showed similar mean denitrification rates in the spring under pulsing conditions (1366 ± 321 mg N m^{-2} h^{-1}) and steady-flow conditions (1009 ± 321 mg N m^{-2} h^{-1}) (Figure 4a). In the high marsh plots, significantly higher ($P < 0.05$) denitrification rates were observed in spring under pulsing conditions (778 ± 92 mg N m^{-2} h^{-1}) than in the spring under steady-flow conditions (328 ± 63 mg N m^{-2} h^{-1}). In edge plots, mean denitrification rates in the spring (395 ± 140 mg N m^{-2} h^{-1}) and summer (531 ± 140 mg N m^{-2} h^{-1}) were higher under pulsing conditions than under steady-flow conditions (269 ± 80 and 227 ± 107 mg N m^{-2} h^{-1}, respectively); however, due to high variability, the differences were not significant.

N_2O/N_2 ratios in transverse gradient

N_2O emissions were, in general, a small percentage of total denitrification from the wetlands (Figure 4b). Low marsh zones showed low N_2O/N_2 ratios with a maximum of 4.5% in autumn 2005 (Figure 4c) and a minimum of 0.15% in spring 2005. In the high marsh plots N_2O/N_2 ratios were more variable, ranging from 1.23% in spring 2004 to 23% in autumn 2005. In edge plots, the highest N_2O/N_2 ratios were observed in autumn 2005 (39%); in general high marsh and edge zones showed higher N_2O/N_2 ratios than did low marsh plots. N_2O/N_2 ratios increased in the cold seasons (autumn and winter) in all plots.

Denitrification under pulsing vs. steady flow conditions

Based on a 3D elevation model described by Zhang and Schwartz (2005), and using water stage data, we were able to calculate the area with standing water (open water and low marsh), the high marsh area, and edge area (Table 2). Using these data and averages of denitrification rates we were able to calculate the mass of nitrogen lost by denitrification for both pulsing year and steady-flow year. The mean mass lost by denitrification was 147 ± 54 kg N yr^{-1} during the pulsing year and 112 ± 41 kg N yr^{-1} during steady flow; these rates were not significantly ($P > 0.05$) different.

Longitudinal and transverse spatial patterns

We evaluated the patterns of denitrification along two spatial gradients—longitudinal, that is, from inflow to outflow in the wetland, and transverse, from edge to deepwater within the wetlands. To evaluate the longitudinal patterns, we average denitrification rates during the whole study period in the different hydrologic zones and group them near the inflow or near the outflow. For this analysis we also include denitrification rates in the open water zones (Figure 5). Mean denitrification rates near the inflow in the open water (613 ± 105 mg N m^{-2} h^{-1}) and low marsh plots (797 ± 127 mg N m^{-2} h^{-1}) were significantly higher ($P < 0.05$) than near the outflow (349 ± 68 and 387 ± 76 mg N m^{-2} h^{-1}, respectively). However, this pattern was not observed in the high marsh or edge plots.

We also investigated denitrification patterns in a transverse gradient from the deepwater center to the shallow edge of the wetlands. Because denitrification in the open water zones was not measured at the same frequency than in other plots, we used only data from the 2005-growing season (May–September) for investigating transverse spatial gradients (Figure 6). We found significant differences ($P < 0.05$) among different zones in these wetlands. The low marsh zone had higher denitrification rates (800 ± 102 mg

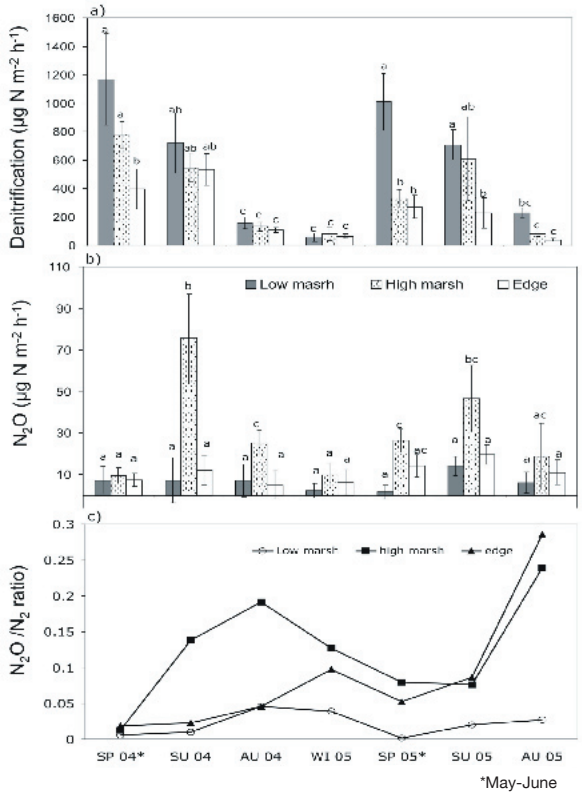

Figure 4. Seasonal a) total denitrification, b) N_2O fluxes, and c) N_2O/N_2 ratios in the transverse gradient in the created wetlands for both pulsing and steady-flow conditions. Values are means, bars represent standard error, and letters indicate significantly different at levels of a = 0.05.

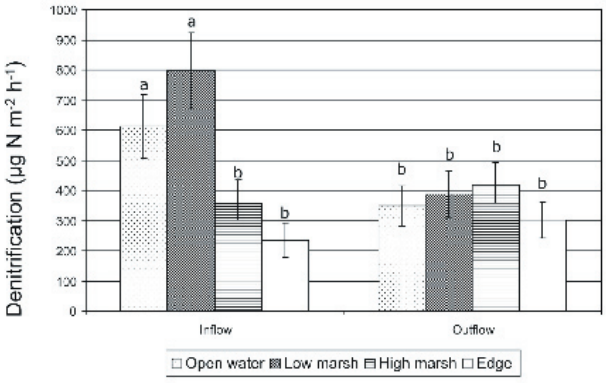

Figure 5. Comparison of denitrification in longitudinal gradient from inflow to outflow in the created wetlands at ORWRP. Values are means, bars represent standard error, and letters indicate significant differences at levels of a = 0.05.

N m^{-2} h^{-1}) compared to those in the high marsh zone (458 ± 87 mg N m^{-2} h^{-1}) and edge zone (315 ± 40 mg N m^{-2} h^{-1}), but not significantly different from the open water zone (584 ± 101 mg N m^{-2} h^{-1}). Denitrification rates in high marsh zones were not significantly different (P > 0.05) than those in the open water or edge zones.

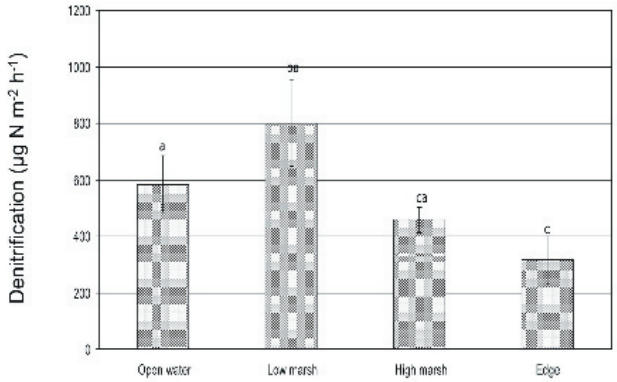

Figure 6. Mean denitrification rates during the growing season (May–September) in the transverse gradient of flooding frequency in the two created wetlands at ORWRP. Values are means, bars represent standard error, and letters indicate significant differences at levels of a = 0.05.

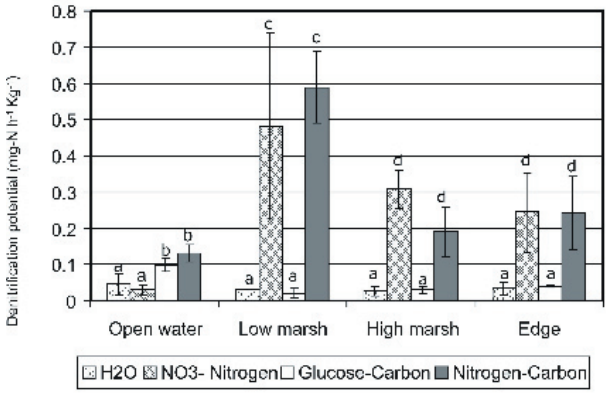

Figure 7. Effect of nitrate and glucose additions on denitrification potential in soils from different hydrologic zones in the two created wetlands at ORWRP. Values are means, bars represent standard error, and letters indicate significant differences at levels of a = 0.05.

Carbon and nitrogen as limiting factors

Besides hydrology, we also investigated the role of carbon and nitrogen as factors limiting denitrification in the different hydrologic zones in these created riverine marshes. We tested the effect of various treatments (1 = H_2O, 2= $N\text{-}NO_3^-$, 3 = C-glucose, and 4 = $N\text{-}NO_3^-$ + C-glucose) on denitrification potential in soil slurries from the same plots where in situ denitrification was analyzed. Denitrification activity measured in this incubation provides a potential rate under no limited conditions and can be used as an index of denitrifying population density (Clement et al., 2002). Absolute values of denitrification activity under these conditions are not informative, per se; however, comparisons of rates under different treatments can reveal denitrification-limiting factors. In this study we found that denitrification rates in soils from the low marsh, high marsh and edge zones increased significantly with nitrate addition (Figure 7). However, no significant increase (P < 0.05) in denitrification activity was observed with the addition of

glucose. A different response was observed in soils from open water zones; in these soils, denitrification did not increase significantly with the addition of $N\text{-}NO_3^-$ or glucose, but a significant increase was observed with the addition of both glucose and $N\text{-}NO_3^-$.

Discussion

Effect of temperature and nitrate on denitrification

Soil temperature was a critical factor controlling denitrification rates in these created riverine wetlands. The effect of temperature on denitrification has been described frequently in the literature for riparian soils; however results are not consistent. Some studies describe significant effects and others have not observed any effect. For example Pavel et al. (1996) found higher denitrification rates in incubations at 19.9°C than at 16.4 or 13.5°C in non-tidal riparian wetland soils. Hefting et al. (2003) found a significant seasonal effect on denitrification rates in the intermediate strip of riparian forested soils in The Netherlands. However, they did not find seasonal effects on denitrification rates in the intermediate strip of grasslands, which had lower denitrification rates.

We observed a significant positive relationship between denitrification rates and soil temperature; this finding agrees with other studies on denitrification in created and constructed wetlands. Teiter and Mander (2005) found that N_2 fluxes correlated significantly with mean top soil temperatures in constructed wetlands treating wastewater in south Estonia, and Poe et al. (2003) found a significant positive correlation between denitrification rates and temperature in constructed wetlands receiving agricultural runoff in North Carolina, USA.

Effect of flood pulse and nitrogen availability

We did not find a significant correlation between nitrate concentrations in the surface water and denitrification rates in the high marsh and edge zones. We believe this was due to the fact that edge and high marsh plots were not permanently inundated, thus NO_3–N may have been generated by soil internal processes (nitrification) as well as by hydrologic fluxes of surface water. Flooding on these plots created anaerobic conditions and both nitrate from the water and soil could be lost by denitrification. After flooding, some micro-anaerobic zones in the soil pores might still have denitrification activity and nitrification might have occurred in the aerobic zones. Nitrate diffusion from aerobic to anaerobic micro-sites might have enhanced denitrification in the summer despite very low nitrate concentration in the surface water. During steady-flow conditions, high marsh zones were saturated but without standing water; these conditions might cause fewer aerobic sites for nitrification thus resulting in less availability of nitrogen in the spring. In the summer, due to high temperatures, evaporation of water from the soils might have caused some micro-aerobic sites in the upper part of the soils and hence sources of nitrates for denitrification in the anoxic layers. High marsh plots had significantly higher nitrate concentrations in 2004 than in 2005 (Hernandez and Mitsch, in press), which indicates that alternative flood and dry conditions (pulses) favored nitrogen availability more than saturated conditions. Denitrification in edge plots was enhanced by flood pulses in the same way as in high marsh plots. However, under steady-flow conditions these plots were dry with more aerobic conditions that resulted in lower denitrification rates. In the steady-flow conditions of 2005 these plots had higher nitrate concentrations than during the spring pulsing period of 2004 (Hernandez and Mitsch, in press), indicating that the surface aerobic conditions favored nitrification and lower denitrification rates, resulting in a net accumulation of nitrates in the soils.

On the other hand, low marsh soils had very low nitrate concentration and high ammonia concentrations due to the anoxic conditions of these permanently flooded soils. It seems that the major nitrate source for denitrification in this zone is nitrate dissolved in water and in some nitrification that might have occurred in the micro-aerobic interface of the sediment-water column and near plant roots (Reddy et al., 1989). In deepwater plots, nitrogen concentrations in water play an important role in controlling denitrification rates. This also explains the higher denitrification rates near the inflow, since it has been well documented that nitrate concentrations in the surface water of these wetlands decrease longitudinally from inflow to outflow (Mitsch et al., 1998, 2005c; Spieles and Mitsch, 2000; Hernandez and Mitsch, in press). We consistently observed high denitrification rates in these deepwater plots in late spring in both years when a combination of warmer temperatures and high nitrate concentration occurred. This combined effect of temperature and nitrate concentration was also found in the Florida Everglades wetland soils with high denitrification enzyme activity during summer when temperature and nutrient loading were high (White and Reddy, 1999).

N_2O/N_2 ratios

Our results showed that permanently flooded low marsh zones had lower N_2O/N_2 ratios of emissions than did intermittently flooded high marsh and edge zones. This may be due to the fact that high marsh and edge zones had more aerobic conditions than did low marsh zones. Nitrous oxide reductase, the enzyme responsible for N_2 production, is more strongly inhibited by oxygen than by reductases involved in N_2O production (Wrage et al., 2001). Nitrate concentrations in high marsh and edge soils were higher than in low marsh soils (Hernandez and Mitsch in press), suggesting that nitrification may have also occurred in these plots. N_2O is also a byproduct of nitrification; therefore, this process may have also contributed to N_2O production (Stevens et al., 1997, Stevens and Laughlin, 1998; Wrage et al., 2001). We were not able to differentiate between N_2O produced by nitrification from N_2O produced by denitrification and this is beyond the scope of our study. We also observed that N_2O/N_2 ratios increased in the cold

months. This means that N_2 production decreases more drastically at low temperatures than does N_2O production; therefore, a major percentage of denitrification end products is N_2O. Laboratory studies with saturated soils have found that the N_2O/N_2 ratio increased when temperature decreased (Bailey and Beauchamp, 1973). Holtan-Hartwing et al. (2002), in lab incubations with soils from Finland, Sweden and Germany, found that low temperatures affected N_2O reductase enzymes to a greater extent than N_2O-producing enzymes (NO_3-, NO_2- and NO reductase), causing higher N_2O/N_2 ratios.

Effect of flood frequency

In general we found higher denitrification rates in permanently flooded plots (low marsh and open water zones) followed by saturated intermittently flooded (high marsh) and dry intermittently flooded (edge plots). This could have occurred because enzymes involved in NO_3 reduction are inhibited by the presence of oxygen (Wrage et al., 2001). It appears that anoxic conditions play an important role in controlling denitrification rates in these riverine wetlands fed with a river rich in nitrates. This pattern has been observed in natural riverine wetlands, salt marshes, and riparian buffer zones. For example, Johnston et al. (2001) found that denitrification potential during summer was higher in zones where standing water covered the soil surface than in zones that were slightly elevated above the water table in natural riverine wetlands in Minnesota. Koch et al. (1992) found that denitrification rates were consistently higher in low marsh zones than in high marsh zones and mudflats in tidal salt marshes in South England. Wigan et al. (2004) also found that potential denitrification activity in the low marsh was greater than in high marsh zones of fringe salt marshes in New England USA. Pavel et al. (1996) found the highest mean denitrification rates in flooded surface horizons compared to terrestrial soils in the Virginia Coastal Plain.

We consistently observed highest denitrification rates in low marsh zones that were permanently flooded and had emergent macrophyte vegetation. These zones showed high organic matter and soluble organic carbon content (Hernandez and Mitsch in press). We believe that the presence of macrophytes in these zones favored the organic matter supply for denitrification. It has also been described that an aerobic environment for nitrification is created near plant roots, enhancing nitrate supply for denitrification in the anoxic zones (Reddy et al., 1989). More recently, it has been described that macrophytes favor nitrate removal in wetlands because macrophyte transpiration stimulates the movement of water into the soil, which facilitates the diffusion of nitrates to the anoxic zones where denitrification occurs (Martin et al., 2003).

Flood pulses enhanced higher denitrification rates in high marsh and edge zones by creating anoxic conditions. However, pulsing conditions did not greatly increase the mass of nitrogen lost by denitrification in these wetlands. We attribute this to the fact that the major proportion of wetland area was permanently flooded, and flood pulses did not affect denitrification rates in these zones.

Carbon and nitrogen as limiting factors

We found that denitrification in low marsh, high marsh and edge zones is limited by nitrate concentrations. The highest denitrification potential was observed in the low marsh zone, probably because the anoxic conditions and the presence of emergent vegetation favored higher denitrifier populations in these permanently flooded zones. In the open water zones, we observed that denitrification was limited by carbon and nitrogen, which suggests that if nitrate concentrations increase, denitrification would not increase in these zones because there is not enough electron supply. Denitrification measured in the field in these permanently flooded sites was not significantly different from rates observed in low marsh plots. This was probably due to the fact that water nitrate concentrations observed in the field were low; therefore, no high organic matter demand was necessary to denitrify them. In other words, under field conditions, these plots had the capacity to carry out denitrification in rates similar to those in the low marsh, but not with higher nitrate concentrations. On the other hand, denitrification rates under field conditions in high marsh and edge plots were lower than low marsh plots, probably due to the absence of permanent anoxic conditions.

Conclusions

Denitrification rates in these created riverine marshes were strongly influenced by soil temperature and hydrologic conditions in the transverse gradient of the wetlands. Permanently flooded (open water and low marsh) zones showed higher denitrification rates than intermittent flooded zones (high marsh and edge). Low marsh plots, which were permanently flooded and had emergent macrophyte vegetation, showed highest denitrification rates in the warmer season (spring and summer); we attributed this to the fact that macrophytes enhance organic matter supply, aerobic micro-environments for nitrification, and diffusion of nitrates to the anoxic zones.

Flood pulses enhanced denitrification in high marsh and edge zones by creating alternate aerobic–anoxic conditions that favored both nitrification and denitrification. Higher denitrification rates in the high marsh and edge zones during flood pulses led to a higher mass of nitrogen lost by denitrification under pulsing conditions than under steady-flow conditions.

Denitrification in the low marsh, high marsh and edge zones was nitrogen limited while denitrification in open water zones was both carbon and nitrogen limited.

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